

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 85106723.1

(51) Int. Cl.⁴: **C 07 F 15/00**
A 61 K 31/28

(22) Date of filing: 31.05.85

(30) Priority: 01.06.84 US 616362

(43) Date of publication of application:
04.12.85 Bulletin 85/49

(64) Designated Contracting States:
DE FR GB IT

(71) Applicant: MASSACHUSETTS INSTITUTE OF
TECHNOLOGY
77 Massachusetts Avenue
Cambridge, MA 02139(US)

(72) Inventor: Lippard, Stephen J.
15 Humboldt Street
Cambridge, MA 02140(US)

(72) Inventor: Bowler, Bruce E.
24 Magazine St. Apt. 3
Cambridge, MA 02139(US)

(74) Representative: Patentanwälte Zellentin
Zweibrückenstrasse 15
D-8000 München 2(DE)

(54) Platinum-intercalative complexes for the treatment of cancer.

(57) Anticancer active compounds are provided by chemical-
ly linking a platinum anticancer drug to an intercalative drug
with a linking group that does not inactivate either drug.
Further on processes for their production and pharmaceutic-
al compositions containing same are disclosed.

May 31, 1985

Eu 85 409 Gr/fr

Platinum-Intercalative Complexes
For The Treatment Of Cancer

This invention relates to novel platinum-intercalative complexes for the treatment of cancer.

Prior to the present invention, there have been available a wide variety of platinum compounds useful as antitumor agents. These available platinum antitumor compounds are quite effective against a variety of tumors. However, they have limited solubility in water, which renders their administration to human patients difficult. In addition, it is common to encounter tumor cell lines which are resistant to these therapeutic platinum compounds, such as cisplatin.

Representative antitumor platinum compounds are disclosed, for example, in U.S. Patents 4,053,587; 4,115,418; 4,140,707; 4,177,263; 4,258,051; 4,339,437 and 4,419,351.

In addition, it is well known that intercalative drugs such as vinblastine and bleomycine are effective antitumor drugs. Intercalative compounds are those which insert themselves between the base pairs of the DNA double helix and may bind

1 to specific sites of the nucleotides forming the DNA. By
binding in this manner, the intercalative drug is believed
to prevent the cellular reproduction of DNA and thereby to
inhibit or prevent further growth of the tumor.

5

It has been proposed by Bagetta et al in Cancer Treatment
Reports, Volume 66, No. 6, June, 1982, to combine cis-dia-
mminedichloroplatinum(II) (cisplatin) with vinblastine and
bleomycin, the latter being an intercalative drug, in order
10 to treat patients afflicted with metastatic malignant mela-
noma. The authors state that the administration of this
combination of drugs appears not to be indicated for general
use, due to the cumulative toxicity of cisplatin. It also
has been reported by Wittes et al, Oncology, Vol. 32, pages
15 202-207 (1975) that there is a synergism noted when cispla-
tin and bleomycin are administered to a patient concomitantly
for cancer treatment.

It would be highly desirable to provide a means for admini-
20 ~~stering antitumor drugs which affords convenient admini-~~
stration and which is at least as effective in the treatment
of cancer as are presently available chemotherapeutic
agents. Furthermore, it would be highly desirable to provide
such a means which is capable of treating a spectrum of
25 different cancers.

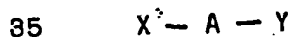
This invention provides novel anticancer drugs comprising a
DNA intercalative drug chemically linked to a platinum anti-
cancer drug in order to form a single molecule which can be
30 administered to a patient. It has been found that the com-
pounds of this invention are more highly water-soluble than
the counterpart unmodified platinum drug, and that they are
toxic toward tumor cells which are resistant to the unmodi-
fied platinum antitumor drug when administered alone. The
35 intercalative drug and the platinum anticancer drug are
joined together by a molecular bridge which does not adver-
sely affect the activity of either the intercalative drug or

1 the platinum drug against tumor cells. Preferred molecular bridges are an alkyl chain, polyamine chain, polyether chain or the like, which can be of variable length and composition.

5 The compounds of this invention are formed from a platinum compound having antitumor activity which includes a site to which a linking chain can be attached such as a reactive ring substituent, i.e., an amino group, a hydroxyl group, a
10 sulfhydryl group, a reactive ring carbon, a ring nitrogen group, or the like. The ring nitrogen group is utilized to bind the linking moiety between the intercalative compound and the platinum compound. Representative suitable platinum compounds include dichloroethylenediamineplatinum(II), cis-
15 -diaminedichloroplatinum(II), 1,2-diaminocyclohexanedi-chloroplatinum(II), cis-diamminemalonatoplatinum(II), or the like.

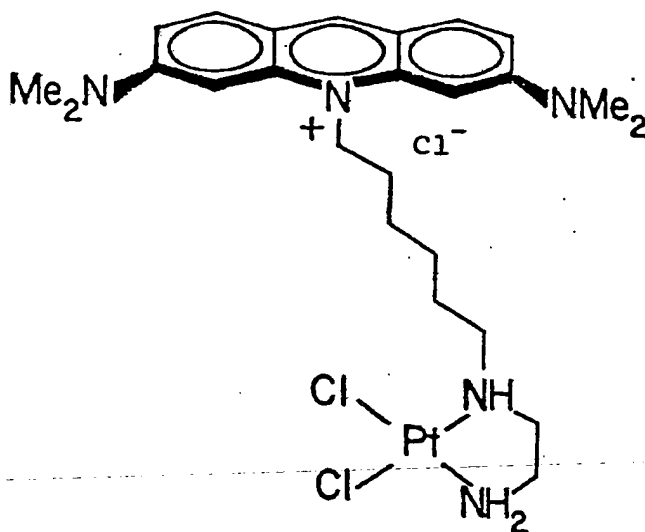
Representative suitable intercalative drugs are those having
20 intercalative activity and having a moiety to which the linking group can be attached, e.g., a ring nitrogen atom. Representative suitable intercalative drugs include acridine orange, 2-methoxy-6-chloro acridine, 9-amino acridine, proflavin or other acridines, adriamycin, daunomycin, ellipti-
25 cine, ethidium bromide, and related phenanthridines.

The compounds of this invention are prepared in a manner which does not adversely affect the intercalative activity of the intercalative drug or the antitumor activity of the
30 platinum drug. Generally the compounds of this invention are prepared by the reaction of an intercalative drug which has a linkable group or atom as an amino or hydroxy group or a ring nitrogen atom with one reactive group of a molecular bridging group of the following formula II



in which X and Y are reactive groups or protected reactive groups of different reactivity and A is an alkyl chain, a

1 polyamine chain or a polyester chain and reacting the other
reactive group - if necessary after removing the protective
group - with the platinum drug. A typical synthesis of a
compound of this invention will be described herein, with
5 reference to acridine orange as the intercalative drug, and
dichloroethylenediamineplatinum(II) as the antitumor plati-
num drug having the formula:



Formula I

25

The starting materials, acridine orange hydrochloride and
6-chloro-1-hydroxyhexane, are reacted under conditions to
protect the hydroxyl group of the 6-chloro-1-hydroxyhexane
with dihydropyran. The chloride group is then substituted
30 with iodide, using the Finklestein-Halide exchange reaction,
which is carried out with NaI/NaHCO₃/acetone. The iodo
compound thereby produced then is condensed with the free
base form of acridine orange in a suitable solvent such as
hot xylene, thereby to cause the quarternization of the ring
35 nitrogen. This reaction yields acridine orange with an alkyl
side chain containing a protected hydroxyl group at the end
of the chain. The protection of the hydroxyl functionality

1 is effected in dilute acid, such as dilute HCl in ethanol.
Thereafter, the hydroxyl group is substituted with bromide
utilizing 48% hydrobromic acid to produce the alkylated
acridine orange with a reactive bromine at the end of alkyl
5 chain. The bromine is easily substituted by ethylenediamine
in a suitable solvent, such as methanol, thereby to produce
an alkylated acridine orange with an ethylenediamine group
at the end of the alkyl chain. This compound then is reacted
with PtI_4^{2-} in a suitable solvent, such as dimethylformamide
10 and water, thereby to effect attachment of the platinum ion
to the ethylenediamine chelate. The two iodides are substi-
tuted with chloride by using a stoichiometric amount of
silver nitrate followed by treatment with HCl to produce the
compound of Formula I.

15 The compound of Formula I is useful as an antitumor drug and
has several advantages over cisplatin. For example, its
aqueous solubility is 20 mg/ml, which is far superior to
that of cisplatin, 3 mg/ml. In addition, the compound of
20 Formula I also has a much greater ability to unwind DNA than
other platinum antitumor drugs, due to the high intercala-
tive affinity of the acridine orange moiety for DNA. Both
the acridine orange and the platinum moieties are biolog-
ically active compounds. Having the two in one molecule
25 enhances the effects of both. It has been found that the
compound of Formula I not only is active against normal
tumor cells, but it is active against cisplatin-resistance
tumor cells. In addition, it has been found that the
compound of Formula I is a photoactive DNA degradation
30 agent.

The following examples illustrate the present invention and
are not intended to limit the same.

35 Suitable dosages for utilizing the drugs of this invention
comprise between about 25 mg/kg body mass and about 2,5
mg/kg. For example, the compound of Formula I can be uti-

1. lized at a dosage of between about 2,5 mg/kg and about 15 mg/kg.

• Example I

5

Synthetic Procedures:

Protected 6-chlorohexanol(II):

- 31,0 ml of dihydropyran (341 mmol) was dissolved in 375 ml of CH_2Cl_2 . 1,36 g of PPTS was added as a catalyst. 26 ml of 10 6-chlorohexanol (227 mmol) was added and the solution was stirred at room temperature for 5 hours. The mixture was then washed in a separatory funnel with 2 x 250 ml of half concentrated NaCl solution with 2 g of NaHCO_3 per 250 ml. The CH_2Cl_2 phase was dried with anhydrous Na_2SO_4 . Removal of 15 the solvent by rotary evaporation yielded 49,8 g of a crude yellow material. Vacuum distillation (<10 torr) yielded a main fraction of 31,7 g (63%, b.p: 99-100°C 10 torr) as a clear liquid.

20 Protected 6-iodohexanol(III):

- All glassware was dried in an oven prior to use. 10 g of II (45,3 mmol) was dissolved in 50 ml of dry distilled acetone. 3,8 g of NaHCO_3 (45,4 mmol) was added to the reaction mixture followed by 20,4 g of NaI (136,1 mmol). This mixture 25 was refluxed under N_2 for 19 hours. The acetone was removed by rotary evaporation.

- The residue was dissolved in deionized H_2O . Two layers formed in the separatory funnel. The mixture was extracted with 2 x 50 ml of Et_2O . The combined ether layers were 30 washed with 50 ml D.I. H_2O (+ 1/2 g NaHCO_3) and then dried with Na_2SO_4 . The solvent was then removed by rotary evaporation and the oil obtained was dried in a vacuum desiccator overnight. Yield: 13,1 g (92,0%).

35 Quaternized Acridine Orange with a Protected Alcohol:

- All glassware was dried in an oven overnight. The xylene was dried over 4 A molecular sieves. The acridine orange free

1 base was dried in a vacuum desiccator overnight. 11,77 g
(37,7 mmol) of III and 65 ml of Xylene were mixed together.
5 g (18,8 mmol) of acridine orange was added as a suspen-
sion. A spatula tip full of NaHCO_3 was added. The mixture
5 was refluxed with vigorous stirring for 5 1/2 hours. The
reaction mixture was cooled and suction filtered. After
washing with Et_2O a bright orange microcrystalline solid was
obtained. After drying in a vacuum desiccator the crude
yield was 8,9 g (83,6%). The product was recrystallized
10 twice from EtOH with Et_2O added to the fog point. 7,96 g
(73,3%) of a bright red-orange microcrystalline solid was
obtained.

Synthesis of Alcohol (VI):

15 5,36 g (9,61 mmol) of the protected alcohol (V) was dis-
solved in 300 ml of 95% EtOH by heating on a steam bath. 2,6
ml of concentrated HCl to make a 0,1M HCl/EtOH solution was
added. The solution was stirred on a steam bath for 2 hours
and then stirred at room temperature for 2 more hours. The
20 solvent was then removed by rotary evaporation producing a
deep red solid. The solid was triturated with Et_2O and then
dissolved in 250 ml of D.I. H_2O . The H_2O solution was washed
with 3 x 50 ml of Et₂O in a separatory funnel. The water
layer was then evaporated by rotary evaporation. The crude
25 yield after drying in a vacuum desiccator was 4,68 g
(97,8%). Recrystallization from 1:1 i-PrOH:MeOH yielded
several crops of crystals ranging from dark red needle-like
crystals to bright red microcrystalline solids. Total re-
crystallized yield: 3,34 g (69,8%).

30

Synthesis of Bromine Compound (VII):

48% HBr was distilled from red phosphorous under N_2 . 1,3 g
(2,63 mmol) of the alcohol VI was added directly to 60 ml of
the freshly distilled 48% HBr. The mixture was stirred on an
35 oil bath under N_2 at 95°C for 4 1/2 hours. The solution was
then poured into 250 ml of cold D.I. H_2O . An immediate light
orange precipitate formed. This was collected by suction

1 filtration through a glass frit. This was followed by wash-
ing with D.I. H_2O and Et_2O . After drying overnight in a
vacuum dessicator, 1.3 g (97%) of VII was obtained. This
material was found to decompose somewhat during recrystal-
5 lization from alcohols. Since the crude solid proved to be
very pure by 1H NMR it was used without further purifi-
cation.

Synthesis of Ligand:

10 The bromine compound (VII) was dried thoroughly in a vacuum
desiccator before the reaction. All glassware was oven dried
before use. 2.80 g of VII (5.5 mmol) was suspended in 200 ml
of dry distilled MeOH. The reaction vessel was flushed with
 N_2 and then 7.36 ml of dry distilled ethylenediamine (110
15 mmol) was added by syringe. The reaction was stirred at $70^\circ C$
under a constant pressure of N_2 . After the first hour all of
VII had completely dissolved. The reaction was stirred 6 1/2
hours total and then most of the MeOH was removed by rotary
evaporation. 40 ml of DMF was added and removed at $55^\circ C$ by
20 rotary evaporation using a vacuum pump. A deep red brown
solid formed in the flask. This was dried in a vacuum
desiccator overnight. The solid was triturated with Et_2O
(3 x 30 ml) and dried in a vacuum desiccator again. Yield:
3.12 g (quantitative).

25

Recrystallization of the Ligand to Produce the Tetra-HCl Salt:

2 g of the crude ligand was suspended in 100 ml of dry
distilled EtOH. This was brought into solution on a steam
30 bath. Dry HCl gas was passed through the solution. A pre-
cipitate initially formed, but this redissolved as more HCl
was bubbled through, producing a much deeper red solution
than originally. The solution was slowly cooled to room
temperature covered with a rubber septum and then cooled in
35 an ice bath. The solid formed was collected by suction
filtration, washed with cold EtOH (200 proof), and dried on
the filter under a fast flow of N_2 . This yielded 1.6 g

1 (77,3%) of the tetra-HCl salt after drying in a vacuum desiccator.

Platinum Diiodo Complex with Ligand (IX):

5 A pH 10 solution of 0,5 g (,848 mmol) of VIII was prepared initially in 10 ml of D.I. H₂O. This was concentrated by rotary evaporation after adding 5 ml of DMF. A white solid was removed and a final 15 ml solution of 2:1 DMF:H₂O was prepared. 0,5 g (1,21 mmol) of K₂PtCl₄ was dissolved in 5 ml
10 of D.I. H₂O. 1,61 g of KI (9,68 mmol) was dissolved in 5 ml of D.I. H₂O. The KI solution was added dropwise to the K₂PtCl₄ solution over 15 minutes, after which the solution was heated at 50°C for 15 minutes. Then 20 ml of DMF was added. The ligand solution was added slowly to the K₂PtI₄
15 solution over 2 hours. DMF was added when needed to keep everything in solution. The solution was stirred overnight at 50°C. The solvent volume was then reduced to a low volume by rotary evaporation. D.I. H₂O was added to precipitate the product. This product was filtered, washed with EtOH and
20 Et₂O, and then dried on a vacuum desiccator overnight.
Yield: 744,7 mg (89,2%) of IX.

Dichloro Platinum Complex X:

300 mg (,305 mmol) of IX was dissolved in 15 ml of DMF.
25 153,7 mg (1,06 mmol) of AgNO₃ was dissolved in 3 ml of DMF. This latter solution was added dropwise to the solution of IX. A heavy whitish precipitate formed. The solution was heated on a steam bath to coagulate the AgI and then stirred 5 minutes more. The solution was then filtered through a
30 Millipore filter (yield: 203,8 mg 95,9% of AgI), stirred for an additional hour covered with foil and then cooled in a refrigerator at 0°C for 1 hour. After filtering through a Millipore filter again the solution was concentrated by rotary evaporation to low volume and 5 ml of DMF and 5 ml of
35 0,4M HCl were added. After sitting overnight the solution was filtered. After removing all but 1-2 ml of DMF by rotary evaporation, the product was precipitated by adding iPrOH.

- 1 The solid was collected by suction filtration and washed with EtOH and Et₂O. After drying in a vacuum desiccator 196,3 mg of X (90,6%) was obtained as a bright red solid.

5

Example II

- 10 Toxicity tests were conducted in mice with the compound of Formula I. A series of culture plates with the cell culture set forth in Table I were exposed to varying concentrations of the compounds to determine the concentration at which cell growth rate decreased by 50% (ID₅₀). The results are set forth in Table I:

15

Table I

Cell Line	ID ₅₀ , Mg/ml
L1210 (leukemia tumor cell line)	0,83
T1815	3,15
L1210 PDD (cis platinum resistant tumor cell line)	4,16

20

- 25 Varying concentrations of the compound of Formula I, i.e., 2,5, 5,0, 10,0, 20,0 and 40,0 mg/kg were each administered to two mice to determine the effect of the compound on the viability of the mice. At 2,5 and 5,0 mg/kg, the mice remained alive. At 10,0 mg/kg, the mice lost weight after 14 days. After 20 and 40 mg/kg, the mice died.

- 30 Mice were implanted with the L1210 cell line interperitonally and thereafter some of the mice were administered with doses of the compounds of formula I ranging from 6,7 to 20 mg/kg. The optimal dose is 15 mg/kg giving a %ILS of 51% versus control mice implanted with L1210 but not treated with the drug. Results are shown in 35 detail in Table II. %ILS values for cisplatin (cisDDP) are also shown in Table II for comparison.

1 Table II

Line	Drug	Dose	Schedule	Mean	ILS
5	L1210/0	Formula I	d 1, 5, 9, 13	20	9,9
				10	12,3
				5	10,6
	CisDDP	3			11,9
					34%
10	L1210/0	Formula I	d 1, 5, 9, 13	15	13,9
				10	11,3
				6,7	11,3
	CisDDP	4,5			23%
					23%
					113%
					51%
15		2,0			11,6
					26%

Treatment with the compound of formula I, of mice implanted with a tumor cell line resistant to cisDDP using doses ranging from 6,7 to 15 mg/kg showed mild activity. These results are shown in Table III.

25 Table III

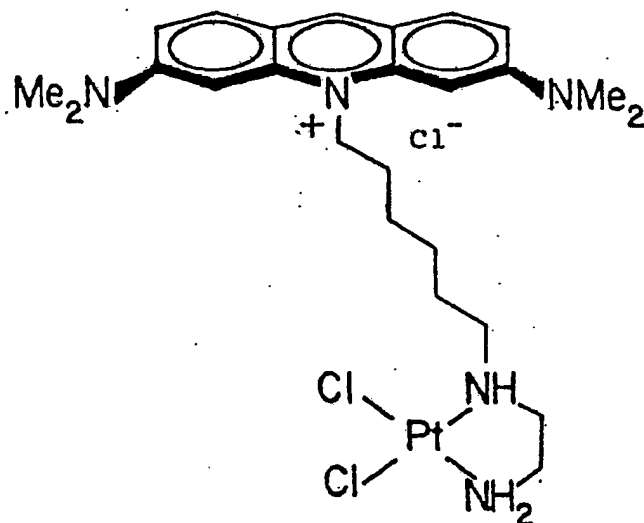
Line	Drug	Dose	Schedule	Mean	ILS
30	L1210/PDD	Formula I	q4dx4	15	12,3
				10	12,1
				6,7	11,4
	CisDDP	4,5			4%
					0
					0
					0
35		2,0		11,0	0

May 31, 1985
Eu 85 409 RZ/fr

Claims:

1. A compound suitable for the treatment of cancerous tumors which comprises an intercalative drug and a platinum drug having antitumor activity linked together by a molecular bridge which does not adversely affect the intercalative activity of the intercalative drug or the antitumor activity of the platinum drug.
2. A compound according to claim 1, in which the linking molecular bridge is an alkyl chain, a polyamine chain or a polyether chain.
3. A compound according to claim 1 or 2, in which the intercalative drug is an acridine or phenanthridine derivative.
4. A compound according to claim 3, in which the intercalative drug is acridine orange, 2-methoxy-6-chloro-acridine, 9-amino acridine, proflavin, adriamycine, daunomycine, ellipticine or ethidium bromide.
5. A compound according to one of claims 1 to 4, in which the platinum drug is dichloro-ethylenediamineplatinum(II), cis-diaminedichloroplatinum(II), 1,2-diaminocyclohexanedichloroplatinum(II) or cis-diaminemalonatoplatinum(II).

6. The compound of Formula I having the formula:



7. A process for the production of compounds according to claims 1 to 6, comprising the reaction of an intercalative drug which has a linkable group or atom as an amino or hydroxy group or a ring nitrogen atom with one reactive group of a molecular bridging group of the following formula II



in which X and Y are reactive groups or protected reactive groups of different reactivity

and A is an alkyl chain, a polyamine chain or a polyester chain

and reacting the other reactive group - if necessary after removing the protective group - with the platinum drug.

8. A process according to claim 7, wherein the reaction of the second reactive group is affected with a diamine compound, which is thereafter chelated with a platinum ion to form the platinum drug.

- 1 9. A process according to claim 8, wherein acridine orange
is reacted with dihydropyrane protected 6-iodo-1-hydroxy-
-hexane, the hydroxygroup is converted to a bromine
5 group, which is condensed with ethylenediamine, which is
thereafter chelated with platinum tetraiodide and the
iodide converted to chloride to yield the compound of
claim 6.
- 10 10. A pharmaceutical composition containing a compound
according to claims 1 to 6 and pharmaceutically accept-
able carriers and adjuvants.
- 15
-
- 20
- 25
- 30
- 35



European Patent
Office

EUROPEAN SEARCH REPORT

0163316

Application number

EP 85 10 6723

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	EP-A-0 054 215 (J. KLOSA)	1	C 07 F 15/00 A 61 K 31/28
X,P	--- CHEMICAL ABSTRACTS, vol. 101, no. 19, 5th-19th November 1984, Columbus, Ohio, USA; E.B. BOWLER et al. "Synthesis and DNA binding and photonic properties of acridine orange linked by a polymethylene tether to (1,2-diaminoethane)dichloroplatinum(II)", page 23, column 2, abstract no. 163301s & J. Am. Chem. Soc., vol. 106, no. 20, 1984, pages 6102-6104	1-3	
A	--- CHEMICAL ABSTRACTS, vol. 99, no. 7, 15th August 1983, Columbus, Ohio, USA; C. MERKEL et al. "Ethidium bromide alters the binding mode of cis-diamminedichloroplatinum(II) to pBR322 DNA", page 25, column 1, abstract no. 47619t Cold Spring Harbor Symp. Quant. Biol., vol. 47, no. ---	1	TECHNICAL FIELDS SEARCHED (Int. Cl.4) C 07 F 15/00
The present search report has been drawn up for all claims.			
Place of search BERLIN		Date of completion of the search 20-08-1985	Examiner KAPTEYN H G
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, r after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	



European Patent
Office

EUROPEAN SEARCH REPORT

0163316

Application number

EP 85 10 6723

Page 2

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	CHEMICAL ABSTRACTS, vol. 97, no. 15, 11th October 1982, Columbus, Ohio, USA; T.D. TULLIUS et al. "Ethidium bromide changes the nuclease-sensitive DNA binding sites of the antitumor drug cis-diamminedichloroplatinum(II)", page 23, column 1, abstract no. 120200q & Proc. Natl. Acad. Sci. U.S.A., vol. 19, no. 11, 1982, pages 3489-3492 -----	I	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 20-08-1985	Examiner KAPTEYN H G
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	